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An improved formaldehyde condensation reaction for the detection of catecholamines on silica gel thin layers*

FALCK AND HILLARP introduced a fluorescence microscopical method for the histochemical demonstration of catecholamines and 5-hydroxytryptamine in which these amines are converted into highly fluorescent end products by exposure to formaldehyde gas[1–4]. Recently, it was shown that formaldehyde condensation of these monoamines also occurs in silica gel, which permits the detection of very small quantities of these compounds by thin layer chromatography[5–7]. One of the difficulties encountered in the chromatographic analysis of these compounds is that they are labile in an oxidizing environment[5]. It is well known that both catecholamines and 5-hydroxytryptamine are readily oxidized at neutral or alkaline pH[8–10] and that silica gel is capable of promoting their conversion into pigments[5], possibly because of its content of heavy metal ions.

The purpose of this study was to elucidate the influence of this oxidation-promoting capacity of the silica gel on the yield of the fluorophores obtained from formaldehyde condensation of easily oxidized amines. When the oxidizing capacity of the thin layer was diminished by adding EDTA (10^{-3} *M*) to the silica gel or when the oxygen tension in the atmosphere was reduced by carrying out the formaldehyde condensation reaction *in vacuo*, the intensity of the light emitted by the catecholamine fluorophores was increased, while that of their 3-methoxylated derivatives and of 5-hydroxytryptamine appeared unaffected (Table I). On the other hand, when the oxidizing capacity of the silica gel was increased by adding ferric chloride (10^{-3} *M*) no formaldehyde-induced fluorescence of catecholamines could be detected whereas that of 3-methoxytyramine, normetanephrine and 5-hydroxytryptamine was un-

TABLE I

FORMALDEHYDE-INDUCED FLUORESCENCE OF BIOGENIC MONOAMINES ON A SILICA GEL THIN LAYER: MINIMUM DETECTABLE AMOUNT (μg) WITH DIFFERENT REACTION CONDITIONS[a]

Thin layers: (A) Silica gel; (B) Silica gel enriched with EDTA; (C) Silica gel enriched with EDTA, formaldehyde condensation *in vacuo*; (D) Silica gel enriched with EDTA, formaldehyde condensation *in vacuo*, and subsequent gentle borohydride treatment.

Compound	*A*	*B*	*C*	*D*
Dopamine	0.03	0.03–0.01	0.01	0.003
Norepinephrine	0.03	0.03–0.01	0.01	0.003
Epinephrine	0.1	0.1–0.03	0.03	0.01
3-Methoxytyramine	0.01	0.01	0.01	0.01
Normetanephrine	0.03	0.03	0.03	0.03
5-Hydroxytryptamine	0.01–0.003	0.01–0.003	0.01–0.003	0.01–0.003

[a] The minimum detectable amount of catecholamines after exposure to formaldehyde gas varies somewhat from one batch of silica gel to another, possibly because of variations in the metal ion content.

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affected. The simplest explanation of these observations is that the reduction in oxidizing capacity serves to protect the catecholamines from oxidative break-down, thus permitting optimal yield of the formaldehyde-induced fluorophores. However, the increased yield of highly fluorescent products is perhaps not only a result of protecting the catecholamines from oxidative degradation but can also be ascribed to modified reaction conditions, which favours the formation of fluorophores with the highest possible fluorescence intensity (quantum efficiency). It is well known that the formation of the fluorophores takes place as a sequence of reactions, one of the steps being that of oxidative dehydrogenation[11,12], and it has recently been established that the resulting monoamine fluorophores are really mixtures of fluorophores of various colours and of varying fluorescence intensity[13]. Conceivably, the formation of each individual fluorophore in the mixture can be enhanced or suppressed by variations in the reaction conditions. This idea is supported by the finding that gentle reduction of the formaldehyde-induced catecholamine fluorophores by spraying with an aqueous solution of sodium borohydride (0.1 %) resulted in an almost 10-fold increase in the fluorescence intensity (Fig. 1). A higher borohydride concentration (1 %) abolished the fluorescence completely. Particularly high fluorescence intensity is obtained when the formaldehyde condensation reaction is carried out after the elimination of oxidation-promoting factors and followed by gentle reduction with dilute borohydride.

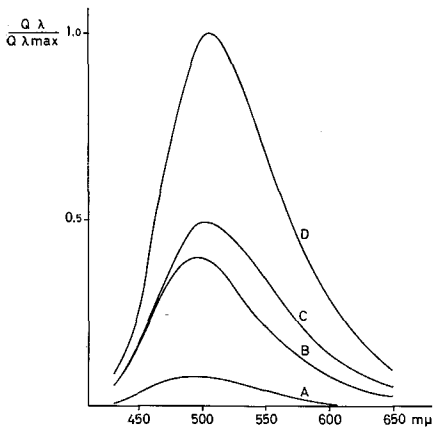

Fig. 1. Fluorescence emission spectra obtained from dopamine on a silica gel thin layer at pH 7.0 after treatment with formaldehyde under various reaction conditions. The analysis was performed in a modified Leitz microspectrograph[14] with a fixed instrument setting for direct comparison of the fluorescence intensities. For explanation of A, B, C and D, see Table I.

Whatever the explanation, it is apparent that the oxidizing capacity of the thin-layer matrix and of the gaseous environment are important factors in determining the eventual fluorescence intensity of the products formed in the condensation reaction between catecholamines and formaldehyde. By modifying the reaction conditions it should therefore be possible to increase the sensitivity of the Falck-Hillarp method and to provide a more selective demonstration of the various groups of monoamines: Anaerobic (or near-anaerobic) conditions can be used to detect both catecholamines

and indolamines, while highly oxidizing conditions reveal indolamines and 3-methoxylated catecholamines only. Apart from being of analytical chemical interest, these principles have also been applied successfully in the histochemistry of biogenic amines[15].

Experimental

Silica gel thin layers were prepared by coating standard histological glass cover slips (24 × 32 mm) with approximately 100 μ Kieselgel H (Merck, Darmstadt). The layer was applied as a slurry consisting of 20 g of silica gel suspended in 50 ml of 0.01 *M* phosphate buffer, pH 7.0. In one series of experiments EDTA (final conc. 10^{-3} *M*) was added to the slurry. The plates were dried at room temperature before used. Aqueous solutions of the amines were spotted onto the plates in volumes of about 0.5 μl. The thin layers were exposed to formaldehyde gas generated from paraformaldehyde (equilibrated in an atmosphere of about 50 % relative humidity) in room air or *in vacuo* at 100° for 30 min. The resulting fluorescence was observed visually over a U.V. lamp (Sterisol, Original Hanau), equipped with a UGI filter. In some cases the plates were sprayed with an aqueous solution of sodium borohydride (0.1 %) and then again observed in U.V. light. Microspectrofluorimetric analysis was performed in a modified Leitz microspectrograph as described in a previous communication[14].

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